5. Persistent EBV Infection in Humanized Mice

EBV infection in immunocompetent hosts is mostly asymptomatic and EBV-infected lymphoblastoid cells with potential to unlimited proliferation are efficiently removed by the virus-specific CTL because they express highly immunogenic proteins such as EBNAs 3A, 3B, and 3C. In EBV latency in humans maintained by T-cell immunosurveillance, EBV reside in memory B cells where all viral protein expression is shut down, rendering them invisible to CTL. Persistent infection reminiscent of EBV latency in humans was reproduced in hu-NOG mice inoculated with low doses of the virus. Majority of hu-NOG mice inoculated with EBV of less than 10^1 TD_{50} remained normal and survived for more than six months without apparent signs of diseases [30]. EBV DNA was detected in the peripheral blood only transiently for the several weeks following infection. When these mice were sacrificed more than six months post-infection, no macroscopic pathological changes were observed, yet a low number of EBER-positive cells were found in the tissues of spleen and lymph nodes. These cells were shown CD20-positive, but their morphology, with rather large cytoplasm, was not consistent with that of resting memory B cells [30]. RT-PCR analysis of RNA obtained from the spleen or liver of these persistently infected mice showed the expression of EBNA1, EBNA2, LMP1, and LMP2A, being consistent with the presence of latency III cells (Yajima *et al.* unpublished results). Thus, persistent EBV infection in hu-NOG mice did not appear to recapitulate all aspects of EBV latency in humans. Since a number of deficiencies have been observed in the functions of B cells in humanized mice [105], reproduction of *bone fide* EBV latency in memory B cells may require more sophisticated humanized mice. Nevertheless, it is an interesting question how immune responses are involved in the induction and maintenance of this persistent EBV infection in hu-NOG mice. Interestingly, EBV DNA level in the peripheral blood fluctuated in a few persistently infected mice and there the rise in EBV DNA level was immediately followed by the increase in $CD8^+$ T cells and subsequent decline of EBV DNA level, suggesting an effective T-cell control of EBV-infected cells [52]. Trials to disrupt this persistent infection by immunosuppressive measures and induce EBV-associated LPD are underway. Cocco and others characterized EBV gene expression, surface marker expression, and hypermutation of immunoglobulin variable region in a single cell level in lymphoid tissues of humanized BRG mice infected with EBV [29]. They could identify EBV-infected cells of all three types of EBV latency (I, II, and III) in specific correlations with the location in the tissue and presence of hypermutation. Thus, the exact route by which EBV establishes latent infection in memory B cells, which is at present unclear, may be clarified in experiments using humanized mice.

6. Characterization of EBV Mutants in Humanized Mice

Among the nine EBV proteins and the two sets of untranslated RNAs expressed in immortalized lymphoblastoid cells, EBNA2, EBNA3A, EBNA3C and LMP1 have been shown to play essential roles in the process of transformation, whereas knocking-out of the EBNA3B gene by homologous recombination did not affect the *in vitro* transforming ability of the virus [67]. Virus replication was not affected either. Nevertheless, as EBNA3B is well conserved in fresh clinical EBV isolates, a critical role for EBNA3B in the life cycle of EBV had been supposed. Recent work by White and others demonstrated that an EBV mutant with its EBNA3B gene knocked out induces more aggressive

LPD in hu-NSG mice, suggesting that EBNA3B functions as a tumor suppressor gene [82]. B cells infected with this mutant virus secreted less T-cell chemoattractant CXCL10 and thereby escaped T-cell mediated killing. EBNA3B may thus help the host to control EBV-induced lymphoproliferation so that the virus should not give a life-threatening harm to the host. These findings were possible only in *In vivo* experiments with humanized mice and points to an important area for their application, namely *In vivo* characterization of virus mutants.

BZLF1 is an immediate-early gene of EBV and acts as a switch from the latent to lytic cycle of EBV infection. Knocking-out of BZLF gene did not affect the virus' ability to transform B cells *in vitro* and the involvement of BZLF1 in lymphomagenesis was not expected until experiments with humanized mice became feasible. Ma and others prepared an EBV recombinant with the BZLF1 gene knocked-out and that with enhanced BZLF1 expression and compared the efficiency of lymphoma genesis in BLT NSG mice [62,63]. The results clearly indicated that BZLF1 enhances lymphoma genesis by inducing abortive lytic infection.

There are a number of EBV genes such as BHRF1 (encoding an Bcl-2-like anti-apoptotic protein) [107], BXLF1 (encoding EBV thymidine kinase) [108], BCRF1 (encoding viral IL-10) [109], loss-of-function mutants of which exhibited no or only minor phenotypic alteration in *in vitro* studies. Examination of these EBV mutants in humanized mice may reveal critical roles for these genes in EBV life cycle and pathogenesis.

7. Mouse Xenograft Models of EBV-Associated T/NK-Cell LPD

Although B cells are the major target of EBV, in a group of diseases termed EBV-associated T/NK-cell LPDs, including CAEBV and EBV-HLH, the virus is mainly found in T or NK cells proliferating oligoclonally or monoclonally. CAEBV is characterized by prolonged IM-like symptoms, unusual patterns of antibody responses to EBV, and elevated EBV DNA load in the peripheral blood [110–112]. In the WHO classification of lymphomas [113], CAEBV corresponds largely with the systemic EBV^+ T-cell lymphoproliferative diseases of childhood. Although monoclonal proliferation of EBV-infected cells implies malignant nature of the disease, chronic clinical time course and absence of morphological atypia in proliferating cells contradicts this notion, and the pathogenesis of this disease is largely unresolved. Overproduction of cytokines by EBV-infected T or NK cells and reacting T cells and macrophages is thought to be responsible for systemic inflammatory symptoms in CAEBV. Although it is still not possible to transform human T and NK cells *in vitro* with EBV to establish immortalized cell lines, the nature of these diseases strongly suggests that in a specific condition EBV can infect T and NK cells and induce their proliferation. EBV infection of T and NK cells has not been reproduced so far in humanized mice and recapitulation of EBV-associated T/NK LPD in mice required xenotransplantation of PBMC derived from patients. Imadome and others transplanted PBMC isolated from patients with CAEBV and EBV-HLH to NOG mice and succeeded in reproducing major features of these diseases including systemic monoclonal proliferation of EBV-infected T or NK cells and hypercytokinemia [99]. Many features were common to CAEBV and EBV-HLH model mice, but the findings of hemorrhagic lesions and extreme hypercytokinemia were unique to the latter model. Importantly, these models revealed an essential role of $CD4^+$ T cells (whether or not infected with EBV) in the *In vivo* proliferation of EBV-infected T and NK cells and depletion of $CD4^+$ T cells by

administrating OKT-4 antibody just following transplantation of PBMC effectively prevented the engraftment of EBV-infected cells [99]. Furthermore, administration of OKT-4 antibody after engraftment of EBV-infected cells reduced peripheral blood EBV DNA load to undetectable level (Imadome and others, unpublished results). These results suggest therapeutic approaches targeting $CD4^+$ T cells may be possible.

8. Future Directions

8.1. Further Analyses on EBV Pathogenesis

EBV is implicated in a variety of diseases (Table 2) and only a minor fraction of them have been recapitulated in humanized mice. Efforts to reproduce the remaining diseases in humanized mice need to be made. Recognition of erosive arthritis resembling RA in humanized mice rationalizes a search for lesions and symptoms of other autoimmune diseases in EBV-infected humanized mice. By varying conditions for EBV infection in humanized mice, including viral dose, viral strain, route of inoculation, timing of infection after transplantation of HSC, as well as modifying the protocol for preparing humanized mice, recapitulation of additional EBV-associated diseases may be possible. As various environmental and host factors are thought to be involved in the pathogenesis of EBV-associated diseases, humanized mice may be a powerful tool for testing the effects of such cofactor candidates *in vivo*. For example, the effects of supposed cofactors for endemic Burkitt lymphoma such as malaria infection and euphorbia plants might be tested in humanized mice. Host genetic factors may be also evaluated in humanized mice; for diseases such as RA in which HLA polymorphism has an influence on pathogenesis, preparing humanized mice with HSC with high-risk polymorphisms may enhance pathogenesis. Similarly, primary immunodeficiency with specific susceptibility to EBV may be reproduced by preparing humanized mice with HSC derived from patients.

8.2. Oral EBV Transmission

EBV is transmitted orally via saliva and initial steps of infection take place in oropharyngeal epithelium and lymphoid tissues just adjacent the epithelium. EBV inoculation to humanized mice so far, however, employed only intravenous or intraperitoneal routes and therefore critical early events in EBV infection may not have been reproduced there. Preliminary trials of oral inoculation of EBV to hu-NOG mice have not been successful (Yajima *et al.* unpublished result). Since no human epithelial cells are present in humanized mice, this result suggests that replication in epithelial cells is an essential step in primary EBV infection. Since oral transmission is a critical initial step in primary EBV infection that may direct later stages of EBV infection in the host, it is highly desirable that this natural route of transmission is reproduced in humanized mice.

8.3. Innate Immune Responses to EBV

Human EBV infection is usually asymptomatic and the symptoms of IM appear only after long incubation period of 3–7 weeks. It is therefore extremely difficult to find individuals currently having acute primary EBV infection in a period suitable for analysis of innate immune responses. In this context, the humanized mouse may be an ideal tool and critical early innate responses to EBV might

be revealed in humanized mice. Although not analyzing early events following infection, one study using NOD/*scid* mice with human fetal thymus xenograft focused on innate immune responses to EBV and demonstrated a role of EBV-induced $CD8^+$ NKT cells in the suppression of tumorigenesis by EBV-associated Hodgkin lymphoma and nasopharyngeal carcinoma cells [114].

8.4. Improving Humanized Mice

Efforts to overcome various limitations in the current humanized mouse models are underway. For example, engraftment of human cells has been improved by introducing human SIRPα transgene to immunodeficient mouse strains or introducing murine CD47 gene to human HSC, thereby avoiding rejection by murine macrophages through improved SIRPα-CD47 signaling [115,116]. Because murine cytokines and growth factors are generally poorly cross-reactive with human receptors, supplementation of human equivalents either by direct injection, introduction of transgenes, or knock-in recombination is expected to improve reconstitution of human immune system components. Indeed, supplementation of human cytokines such as GM-CSF, IL-4, M-CSF, IL-7, IL-15, and EPO has been reported to improve the development and/or maintenance of certain human immune system components [117]. These improved protocols, as well as introduction of human MHC transgenes described above, will eventually realize humanized mice with the capacity of immune responses comparable to those in humans. Evaluation of vaccine candidates, including that for EBV, may become feasible with these improved humanized mice.

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Conflict of Interest

The authors declare no conflict of interest.

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CASE REPORT

Sequential monitoring of serum IL-6, TNF-α, and IFN-γ levels in a CAEBV patient treated by plasma exchange and immunochemotherapy

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Abstract We report the case of a female patient with chronic active Epstein–Barr virus infection (CAEBV) accompanied by hemophagocytic syndrome (HPS). On admission, she presented with severe liver dysfunction and disseminated intravascular coagulation with elevation of serum IL-6, TNF-α, and IFN-γ levels. Plasma exchange (PE) followed by immunochemotherapy with prednisolone, cyclosporine A, and VP16 was performed. PE decreased serum cytokine levels dramatically and improved liver function. Following immunochemotherapy, CAEBV became inactive. Four months after discharge, however, CAEBV relapsed with HPS, and serum cytokine levels were extremely elevated again. There was no response to immunochemotherapy, and the patient died 1 day after admission. We examined the cytokines in five additional untreated-CAEBV patients and determined that they were elevated above the normal level in all patients. These results suggest that inflammatory cytokines may have roles in the development of CAEBV, and that their depletion can be an effective treatment for this disease.

Keywords Chronic active Epstein–Barr virus infection · IL-6 · TNF-α · IFN-γ · Hemophagocytic syndrome

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Introduction

Chronic active Epstein–Barr virus infection (CAEBV) is a relatively rare lethal disorder characterized by sustained infectious mononucleosis-like symptoms accompanied by clonal proliferation of EBV-infected cells which are T or NK cells [1]. Since the disease ultimately progresses to lymphoma or leukemia, CAEBV is classified as one of the mature T- and NK-cell neoplasms, according to the World Health Organization (WHO) classification of hematopoietic neoplasms [2]. In addition, CAEBV also has aspects of severe inflammatory diseases with fever, lymphadenopathy, liver damage, vasculitis, and hemophagocytic syndrome (HPS), which can result in death [3]. To manage and treat CAEBV, therefore, it is necessary to control severe inflammation.

Inflammatory cytokines such as IL-6, TNF-α, and IFN-γ, which induce inflammation itself, were elevated in EBV-associated HPS [4, 5]. They were also increased in CAEBV at the disease onset irrespective of accompanying HPS and suspected to contribute to disease development [6]. There has been, however, no report that monitored them during the clinical course of CAEBV. Here, we examined the serum levels of these cytokines longitudinally in a CAEBV patient treated by plasma exchange (PE) and immunochemotherapy. Using the results, we discuss the role of the cytokines in the disease.

Case report

A 64-year-old female was transferred to our hospital for high fever, liver dysfunction, and disseminated intravascular coagulation (DIC). She suffered from unexplained skin eruptions and edema for several years and with sore throat and low grade fever for more than 3 months before admission. A few days prior to admission, she developed general fatigue, high fever, and severe liver dysfunction with jaundice. On admission, she presented with pancytopenia and lymphadenopathy. Her anti-EBV antibodies were 1:160 for anti-VCA-IgG and 1:10 for anti-EA-DRIgG. Anti-VCA-IgM was undetectable, and the titer of anti-EBNA was 1:40. EBV DNA copy number in peripheral blood (PB) was elevated to 2.3 $\times$ 10^5 copies/μg DNA. The flow cytometry analysis of the PB mononuclear cells (PBMCs) was shown in Fig. 1a. T cells mainly consisted of CD4- and CD8-positive cells with expression of CD2 as well as CD5. To determine the phenotype of the EBV-infected cells, PBMCs were examined according to the methods described in the previous report [7] and were found to be CD8-positive lymphocytes. The clonal

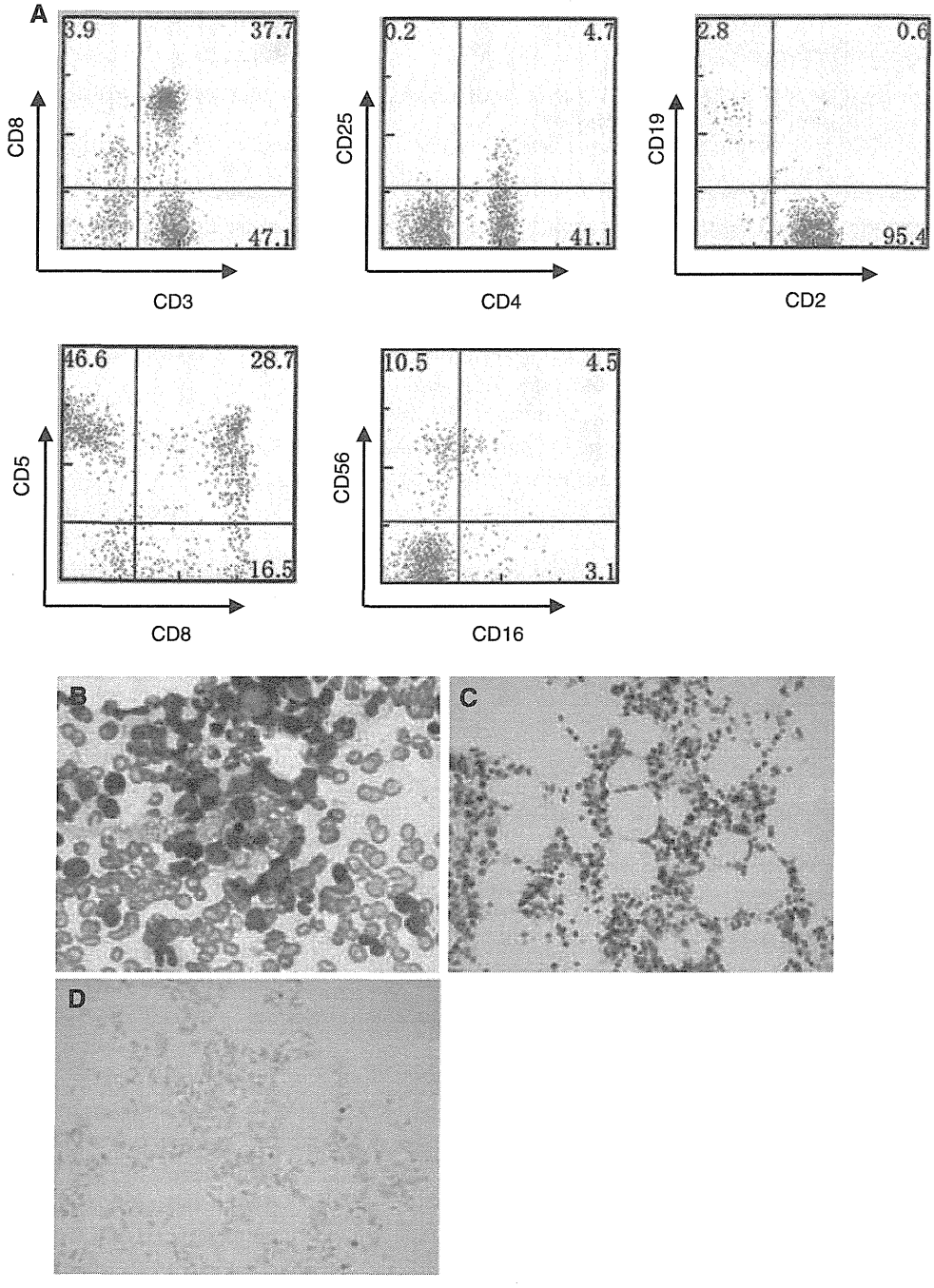


Fig. 1 **a** Analysis of peripheral blood mononuclear cells by flow cytometry at disease onset. **b–d** Specimen of the bone marrow. **b** May-Giemsa staining showing severe hemophagocytosis (×400). **c** Anti-CD8 antibody staining. The infiltration of CD8-positive cells is detected (×200). **d** In situ hybridization of EBER. The infiltration of EBER-positive cells is detected (×200)

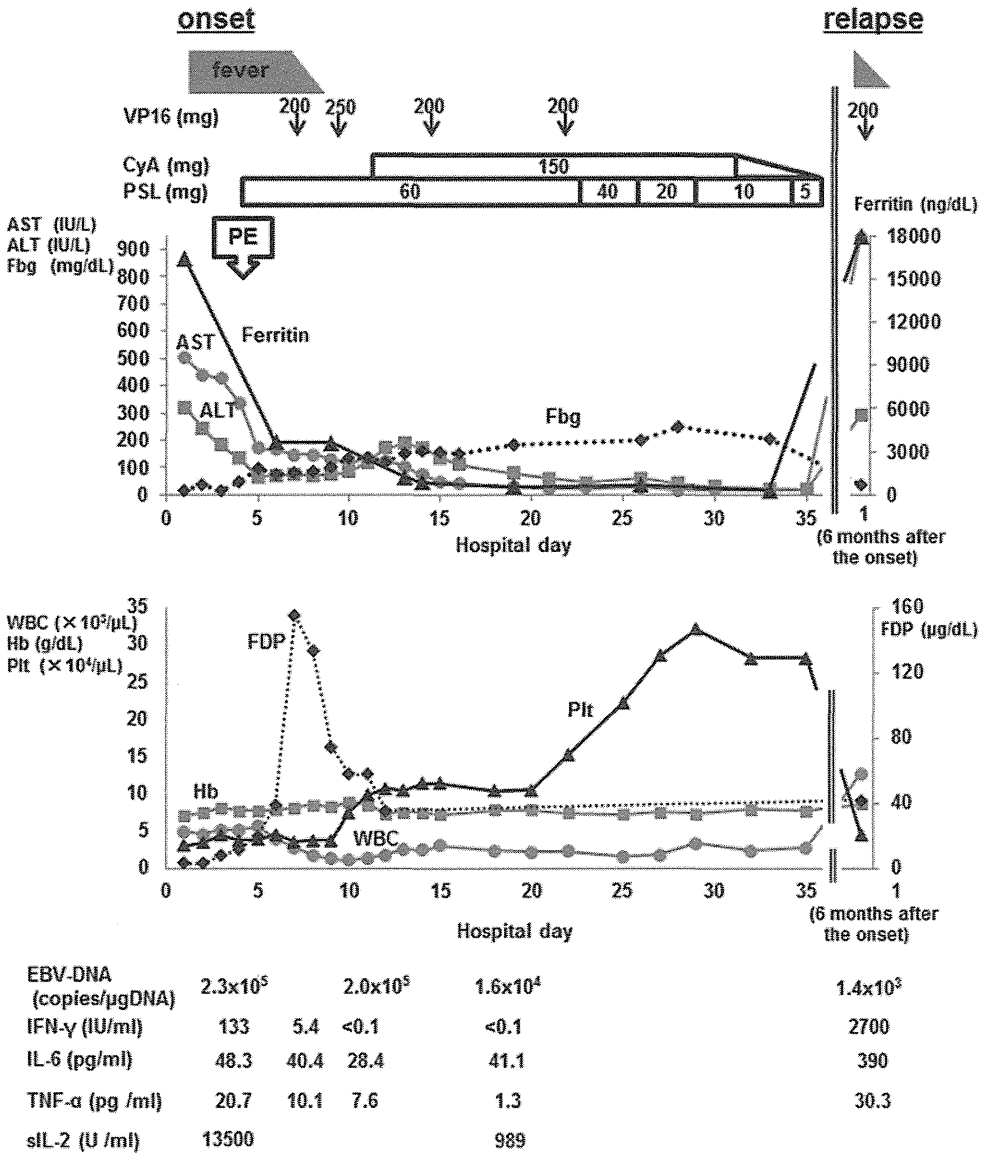
proliferation of the EBV-infected cells was detected by Southern blot analysis for EBV-terminal repeat. Bone marrow revealed CD8-, and EBV-positive lymphocytic infiltration and hemophagocytosis (Fig. 1b–d). According to the diagnostic criteria of CAEBV [8] and HPS [9], we arrived at a diagnosis of CAEBV accompanied by HPS.

Her clinical course is shown in Fig. 2. PE was performed for liver dysfunction and hemophagocytosis followed by immunochemotherapy comprising prednisolone, cyclosporine A, and VP16 [10]. After PE, AST, ALT, and Ferritin were drastically decreased and immunochemotherapy made her disease inactive by resolving fever and improving DIC. After her disease became inactive, she was discharged from the hospital. While preparing hematopoietic stem cell transplantation (HSCT), we gave her the same immuochemotherapy monthly. However, HPS relapsed 4 months after discharge and she died due to severe liver damage and DIC.

During the course, we sequentially examined serum IL-6, TNF-α, and IFN-γ levels. As shown in Fig. 2, they were markedly increased at disease onset, decreased drastically after PE. The cytokines revealed fulminant increase at the relapse, whereas EBV-DNA in the peripheral blood decreased rather than increased. We also examined the cytokine levels in other patients with an active CAEBV disease but without HPS (Table 1). Their EBV-infected cells were T- or NK-cell. As shown in the table, the levels were abnormally high in these patients, but they were not as high as those of the present case.

Discussion

Although CAEBV is classified as a lymphoid malignancy according to the WHO classification, it has many features



EBV-DNA (copies/μgDNA)	2.3×10^5		2.0×10^5	1.6×10^4	1.4×10^3
IFN-γ (IU/ml)	133	5.4	<0.1	<0.1	2700
IL-6 (pg/ml)	48.3	40.4	28.4	41.1	390
TNF-α (pg /ml)	20.7	10.1	7.6	1.3	30.3
sIL-2 (U /ml)	13500			989	

Fig. 2 The clinical course of the present patient. *CyA* cyclosporine A, *PSL* prednisolone, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *PE* plasma exchange, *FDP* fibrin/fibrinogen degradation products, *Fbg* fibrinogen, *EBV* Epstein–Barr virus. Normal ranges of serum IFN-γ, IL-6, and TNF-α were <0.1 IU/mL, <4.0 and 0.6–2.8 pg/mL, respectively

Table 1 The serum concentration of the inflamatory cytokines at the onset in chronic active Epstein–Barr virus infection patients

Case number	Age/gender	EBV copy number of the PB (copies/μg DNA)	The EBV-infected cells	IFNγ (EIA) (IU/ml)	IL6 (pg/ml)	TNF-α (pg/ml)
1	25/F	7.0×10^4	CD4	10.2	66.7	2.4
2	40/M	1.7×10^3	CD4	0.7	28.4	3.0
3	64/F	1.0×10^4	CD4	2.7	16.0	5.5
4	23/F	8.6×10^4	CD56	6.0	161.0	7.2
5	48/F	3.2×10^4	CD56	0.7	16.9	3.4
Present case	64/F	2.3×10^5	CD8	133.0	48.3	20.7

EBV Epstein–Barr virus, *PB* peripheral blood, *EIA* enzyme immunoassay, *M* male, *F* female

that are atypical of lymphoma, such as poor dysplasia of EBV-infected cells, low incidence of solid tumors, and low response to cell cycle-dependent chemotherapy. In addition, we recently found that EBV-infected cells did not establish disease when transplanted alone into NOD/Shi-scid/IL-2Rγ-null strain mice [11], whereas many cancer cells can successfully engraft in this context. On the other hand, CAEBV has aspects of inflammatory disease: fever and polyclonal gammopathy are characteristic findings and may be accompanied by an autoimmune disease [12–15]. In addition, some CAEBV patients develop HPS, a severe inflammatory condition that can be lethal. Thus, control of inflammatory conditions is an indispensable part of the CAEBV treatment.

In this study, we demonstrated that serum IL-6, TNF-α, and IFN-γ levels, which can induce inflammation, were elevated in CAEBV patients irrespective of EBV-infected cell phenotypes and accompanying HPS. Cohen et.al [6] previously reported that serum IL-6, TNF-α, and IFN-γ levels at the onset were elevated in CAEBV patients in the USA. However, the main phenotype of EBV-infected cell in the report was B cell (11/19), and the number of T- or NK-cell type which is dominant in Japan was only 4. Our report demonstrated that IL-6, TNF-α, and IFN-γ levels were also elevated in CAEBV of T- or NK-cell type. Furthermore, these cytokines decreased with successful treatment and increased again with disease relapse in the present case. They may parallel the clinical course and play roles in development of CAEBV. On the other hand, EBV-DNA copy number at the relapse was lower than that in remission. Kimura et al. [16] reported that elevated EBV-DNA copy number in the PBMCs was useful for diagnosis of CAEBV. However, there has been no report regarding the titer and the disease gravity in CAEBV. Our result suggests that they do not parallel each other. Further study should be added to evaluate the relation between the cytokines or EBV-DNA in PB and the disease status.

In vitro, EBV-infected T or NK cells secrete inflammatory cytokines. Roncella et al. [17] detected transcription of IL-6, TNF-α, and IFN-γ in EBV-positive gamma–delta T cell lymphoma cells. Lay et al. [4] also reported that TNF-α and IFN-γ are elevated in the sera of EBV-positive T cell lymphoma patients, as well as in the supernatant of EBV-infected T cell lines, and contribute in combination to the development of phagocytotic activities of co-cultured monocytic cell line, U937. Kanno et al. [18] showed that TNF-α is synthesized and secreted by the EBV-positive NK-cell lines SNK1 and SNK6, and regulates their adhesion to endothelial cells. Based on these observations, we suggest that IL-6, TNF-α, and IFN-γ are produced by EBV-positive cells and may be important contributors to the development and severity of CAEBV disease.

The optimal chemotherapy for CAEBV has not been established. Currently, the only effective strategy to eradicate the infected cells is stem cell transplantation [19]; however, this is not a trivial procedure and not all patients are candidates for this treatment. It is strongly needed to identify an optimal treatment for CAEBV, especially for cases with multiple organ failure due to severe inflammation or HPS as seen in the present case. The effect of PE on CAEBV has not been discussed up to date. In the present case, PE decreased the levels of cytokines and had effects on HPS as well as on CAEBV. These results also indicated that depletion of cytokines was effective for control of the disease. Neutralizing antibodies or inhibitors are available for the cytokines which were elevated in the present case. The in vitro study by Lay et al. demonstrated that anti-TNF-α antibody suppressed phagocytotic activities of U937 cells induced by EBV-infected T cell line [17]. Actually some case reports have indicated that TNF-α-inhibitors, such as infliximab or etanercept, might be effective for control of HPS [20, 21]. Further study should be added to clarify roles of the cytokines and to develop the possibility of anti-cytokine treatment in CAEBV.

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Medical and Dental University Hospital approved this study, and written informed consent was obtained from the patient.

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INTERNAL MEDICINE

CASE REPORT

Recurrence of Chronic Active Epstein-Barr Virus Infection from Donor Cells after Achieving Complete Response Through Allogeneic Bone Marrow Transplantation

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Abstract

We report the case of a 35-year-old woman with chronic active Epstein-Barr virus (EBV) infection (CAEBV). She underwent allogeneic bone marrow transplantation (BMT) from an unrelated male donor and achieved a complete response. However, her CAEBV relapsed one year after BMT. EBV-infected cells proliferated clonally and revealed a 46XY karyotype. In addition, the infecting EBV strain differed from that detected before BMT. These findings indicated that her disease had developed from donor cells. This is the first report of donor cell-derived CAEBV that recurred after transplantation, suggesting that host factors may be responsible for the development of this disease.

Key words: chronic active Epstein-Barr virus infection, bone marrow transplantation, systemic lupus erythematosus

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Introduction

Epstein-Barr virus (EBV) can be detected not only in B-cell tumors but also in T- and NK-cell tumors, which are known as EBV-positive T/NK-cell lymphoproliferative diseases (EBV-T/NK-LPDs). EBV-T/NK-LPDs comprise extranodal NK/T-cell lymphoma nasal type (ENKL), aggressive NK-cell leukemia, and chronic active EBV infection (CAEBV). CAEBV is a rare disorder accompanied by the clonal proliferation of EBV-infected cells (1). Its T-cell infecting type is designated as "EBV-positive T-cell lymphoproliferative disease of childhood" in the WHO classification revised in 2008 (2). However, adult-onset cases have been reported (3, 4).

The pathogenesis of CAEBV is assumed to be due to the EBV infection of T or NK cells followed by their immortalization and expansion. However, the mechanisms responsible for the clonal expansion of infected cells remain unclear.

We report here the case of CAEBV in a female patient. In spite of achieving a complete response (CR) after bone marrow transplantation (BMT), CAEBV recurred. At recurrence, the infected cells were clonally proliferating donor cells, and the infecting virus differed from that originally causing the disease. We describe her clinical course and discuss the possible pathological mechanism responsible for the recurrence.

Methods

The detection and isolation of infected cells (5) and sequence analysis for *perforin* (6) were performed as de-

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scribed previously. For the sequence analysis of the variable region of *lmp1*, the genomic DNA extracted from infected cells was amplified by PCR. The following primers were used: 5-AAGGGAGTGTGTGCCATTAAG-3 (fwd) and 5-A CCCCCACTCTGCTCTCAA-3 (rev); their nucleotide positions in B95.8 (Genbank No.V01555) were 168052-168073 and 168619-168601, respectively. The conditions for PCR reactions were as follows: 94℃ for 5 minutes, 94℃ for 15 seconds, 60℃ for 30 seconds, and 72℃ for 60 seconds; 35 cycles. The amplicon was directly sequenced using the same primers. The ethics committee of Tokyo Medical and Dental University Hospital approved this study, and written informed consent was obtained from the patient.

Case Report

A 35-year-old woman developed fever and cervical lymphadenopathy and was transferred to our hospital. She had systemic lupus erythematosus (SLE) for the previous 13 years and was receiving prednisolone (PSL) at 5 mg/day. Her anti-EBV antibodies on admission were 1:20,480 for anti-VCA-IgG and 1:1,280 for anti-EA-DRIgG, which were extremely elevated. Anti-VCA-IgM was undetectable, and the titer of anti-EBNA was 1:40. EBV DNA copy numbers in peripheral blood (PB) were elevated to 1×10^7 copies/μg DNA.

EBV-positive T-cell lymphoproliferative disease was diagnosed by cervical lymph node biopsy (Fig. 1A). Infiltrating cells were positive for CD8, Granzyme B, and EBER (Fig. 1B-D). In addition, activated $CD8^+$ cells were increased in the PB (Fig. 1E). These cells were EBV-positive; they were clonally similar to those in the lymph node, which involved a *TCRJβ1* gene rearrangement (Fig. 1F-H).

Chemotherapy was administered followed by BMT as described by Koyama et al (7). The donor was a 29-year-old unrelated male. His HLA type was A2 (0207) / A26 (2602), B46 (1501) / B62 (4601), and DR14 (1403) / DR14 (1406). The patient's type was A2 (0207) / A26 (2602), B46 (1501) / B62 (4601), and DR14 (1401) / DR14 (1401). Their serologic HLA types were identical, whereas the DNA types displayed disparities in 2 HLA-DR alleles.

The conditioning regimen for transplantation comprised fludarabine (37.5 mg/m^2 intravenously, once daily from days -6 to -2), melphalan (60 mg/m^2 intravenously, once daily from days -6 to -5), and total body irradiation (4 Gy in 2 fractions on day -1). Cyclosporine (3 mg/kg, from day -1) and short-term methotrexate (5 mg, 10 mg, and 10 mg on days 1, 3, and 6, respectively) were administered for the prophylaxis of acute graft-versus-host disease. Engraftment was confirmed 1 month after BMT, and the EBV genome in PB became undetectable after 2 months and remained so for nearly 12 months.

Although graft-versus-host disease had not developed, administration of low-dose corticosteroid (hydrocortisone, 10 mg/day) was continued to compensate for her endogenous cortisol deficiency due to the long-term administration of PSL. One year later, her EBV DNA level began to increase and reached 1.7×10^4 copies/μg DNA. Three years after BMT, it was 1.0×10^5 copies/μg DNA, and the number of CD8-positive cells had increased among her PB mononuclear cells (PBMC; Fig. 2A).

Infected cells in PB were investigated again; these were identified as CD8-positive T cells. Their clonality was confirmed by detecting a *TCRJβ1* gene rearrangement, which revealed a difference from the original (Fig. 1I). EBV-infected cells (Fig. 2B) and a lymphoblastoid cell line (LCL) established from the patient's PBMC soon after engraftment (Fig. 2C) had XY karyotype, confirming that these were donor cells. Furthermore, sequence analysis of the variable region of *lmp1* showed that the infecting virus differed from that detected in CD8-positive cells before BMT and was identical to that detected in LCL (Fig. 2D). Although we did not examine whether the donor was seropositive for EBV, the virus obtained from LCL might have been of donor origin.

Liver dysfunction developed gradually 4 years after BMT. Liver biopsy was performed, and a significant sinusoidal infiltration of atypical cells (CD8- and EBV-positive) was detected (Fig. 3A-C). Her PBMC retained the 46XY karyotype (Fig. 3D) and mainly comprised activated CD8-positive cells. In addition, CD4-positive cells were detected (Fig. 3E).

The EBV DNA copy numbers, the chimerisms of nucleated cells and lymphocytes, and the percentage of CD4- and CD8-positive cells in peripheral blood are summarized in Table 1. The chimerism maintained the donor type during the clinical course. An abnormal XXYY clone suggesting donor origin appeared 4.5 years after BMT as the disease progressed. From these results, the diagnosis of CAEBV, which developed from donor cells infected with a different virus, was confirmed.

Discussion

The mechanisms responsible for CAEBV development have not been elucidated. Some investigators reported that EBV-infected T or NK cells could be detected during primary infection (8, 9), indicating that EBV could infect these cells under a high level of viral load. However, some factors leading to disease development may exist because CAEBV shows a marked geographic preference for East Asia. Although the strains identified in the present patient before and after BMT were not identical, the relationship between strains and disease development needs to be investigated. In addition, a patient's genetic background may be involved. In our patient, recurrence after BMT underlines the importance of non-hematological factors for disease development.

According to Ohshima et al, following infection with EBV, T, or NK cells can undergo poly-, oligo-, or monoclonal expansion, resulting in CAEBV (10). For the expansion of EBV-infected T or NK cells, suppression of cytotoxic T-cell (CTL) activity may play an important role. Sugaya et al